

UTILITY PATENT APPLICATION TRANSMITTAL

(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
NA-1151

Total Pages in this Submission

TO THE ASSISTANT COMMISSIONER FOR PATENTS

Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

Method for the Purification and Aqueous Fiber Spinning of Spider Silks and Other Structural Proteins

and invented by:

Charlene Mello, Steven Arcidiacon, MichelleM. Butler

If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: _____

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Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 25 pages and including the following:
 - a. ☒ Descriptive Title of the Invention
 - b. ☐ Cross References to Related Applications (if applicable)
 - c. ☐ Statement Regarding Federally-sponsored Research/Development (if applicable)
 - d. ☐ Reference to Microfiche Appendix (if applicable)
 - e. ☒ Background of the Invention
 - f. ☒ Brief Summary of the Invention
 - g. ☒ Brief Description of the Drawings (if drawings filed)
 - h. ☒ Detailed Description
 - i. ☒ Claim(s) as Classified Below
 - j. ☒ Abstract of the Disclosure

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Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*

- a. ☒ Formal Number of Sheets 18
- b. ☐ Informal Number of Sheets _____

4. ☒ Oath or Declaration

- a. ☒ Newly executed *(original or copy)* ☐ Unexecuted
- b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
- c. ☒ With Power of Attorney ☐ Without Power of Attorney
- d. ☐ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application,
see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*

The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche *(Appendix)*

7. ☐ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all must be included)*

- a. ☐ Paper Copy
- b. ☐ Computer Readable Copy *(identical to computer copy)*
- c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & document(s))*

9. ☐ 37 CFR 3.73(B) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☒ Information Disclosure Statement/PTO-1449 ☒ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing

☐ First Class ☒ Express Mail *(Specify Label No.):* EM576738700US

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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)

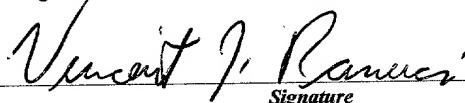
16. ☐ Additional Enclosures (please identify below):

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	18	- 20 =	0	x \$18.00	\$0.00
Indep. Claims	3	- 3 =	0	x \$78.00	\$0.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$690.00
OTHER FEE (specify purpose)					\$0.00
TOTAL FILING FEE					\$690.00

- ☐ A check in the amount of _____ to cover the filing fee is enclosed.
- ☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 19-2201 as described below. A duplicate copy of this sheet is enclosed.
- ☐ Charge the amount of \$690.00 as filing fee.
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- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).


Signature

Dated: JAN. 20, 2000

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CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)Applicant(s): **Mello et al**

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NEW

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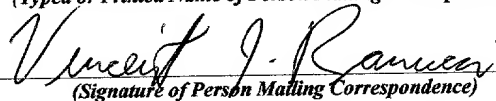
NEW

Invention:

Methods for the Purification and Aqueous Fiber Spinning of Spider Silks and Other Structural ProteinsI hereby certify that this **UTILITY PATENT APPLICATION***(Identify type of correspondence)*

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under

37 CFR 1.10 in an envelope addressed to: The Commissioner of Patents and Trademarks, Washington, D.C.

20231-0001 on **JAN. 20, 2000**
*(Date)***Vincent J. Ranucci***(Typed or Printed Name of Person Mailing Correspondence)**(Signature of Person Mailing Correspondence)***EM576738700US***("Express Mail" Mailing Label Number)***Note: Each paper must have its own certificate of mailing.**

METHODS FOR THE PURIFICATION AND AQUEOUS FIBER SPINNING OF SPIDER SILKS AND OTHER STRUCTURAL PROTEINS

STATEMENT OF GOVERNMENTAL INTEREST

5 The present invention may be used or licensed by the United States Government
for Governmental purposes without the payment of any royalty.

FIELD OF THE INVENTION

10 The present invention relates to methods for purifying and spinning spider silks
and other structural proteins. Specifically, organic acids are used to lyse recombinant
cells or other biological samples (such as non-recombinantly derived cells), and
significantly enrich the purity and yields of structural proteins by hydrolyzing many of
the macromolecules, while leaving the structural proteins intact. In the case of silk
15 proteins, the resulting lysate is further purified by ion-exchange or affinity
chromatography and processed into an aqueous-based mixture for fiber spinning.

BACKGROUND

20 Spiders produce a number of silks for different functions and are therefore useful
organisms to produce a variety of structural proteins. The structural fibers of the golden
orb-weaver spider (*Nephila clavipes*), are extremely strong and flexible, and are able to
absorb impact energy from flying insects without breaking. Dragline silk fibers dissipate
energy over a broad area and balance stiffness, strength and extensibility. In addition,
silk proteins have very low antigenicity. Therefore, silk fibers are well suited for light
weight, high performance fiber, composite and medical applications. The composition of
25 these proteins is mainly glycine, alanine, and other short side chain amino acids, which
form anti-parallel beta-pleated sheets by hydrogen bonding and hydrophobic interactions;
Lucas et al., Discovery 25:19 1964. Many spider silks are resistant to digestion by
proteolytic enzymes; *Tillinghast and Kavanaugh*, Journal of Zoology 202:212 1977, and
insoluble in dilute acids and bases; *Mello et al.*, American Chemical Society Symposium
30 Series 544, Silk Polymers: Materials Science and Biotechnology pp 67-79, 1995. Spiders
are not capable of producing sufficient quantities of proteins to enable a practical use of
their potential. To solve this problem, recombinant spider silks have been expressed in

E.coli; Arcidiacono et al., Applied Microbiology and Biotechnology 49:31 1998; Fahnestock and Irwin, Applied Microbiology and Biotechnology 47:23, 1997; Fahnestock and Irwin, Applied Microbiology and Biotechnology 47:33 1997; Lewis et al., Protein Expression and Purification 7:400, 1996; Prince et al., Biochemistry 34:10879 1995. However, the purification and preparation of a protein for fiber spinning has been particularly difficult due to the solubility characteristics and unique properties of spider silk and other structural proteins.

Native *Nephila clavipes* spider dragline fiber has been partially solubilized in hexafluoroisopropanol (HFIP) and dried to a film. A 2.5 % (w/w) solution of the film in HFIP was used for spinning; Jelinski et al., Macromolecules 31:6733 1998. The spinning was conducted with a syringe pump at 6uL/s by forcing the HFIP solution through the spinneret into a coagulation bath.

Affinity chromatography has been used for purification by binding to an engineered tag in the recombinant protein while washing away bacterial proteins; Arcidiacono et al., Applied Microbiology and Biotechnology 49:31 1998; Fahnestock and Irwin, Applied Microbiology and Biotechnology 47:23 1997; Lewis et al., Protein Expression and Purification 7:400 1996; Prince et al., Biochemistry 34:10879 1995. One commonly used tag is a hexa-histidine tag, that binds with high affinity to a nickel affinity resin. After washing away the bacterial proteins, the tagged recombinant protein can be eluted from the resin. There are several disadvantages to this method: 1) large volumes of denaturing buffers are required, involving multiple steps and time; 2) not all target protein is recovered; 3) other bacterial proteins remain, often requiring additional purification (i.e., high-performance liquid chromatography (HPLC)); 4) the method is not easily scaled-up; 5) and the presence of an affinity tag on the recombinant protein may increase its antigenicity and interfere with the necessary molecular alignment required for high strength fibers. Accordingly, there is a continuing need to develop new methods for the purification of structural proteins, spinning of silk fibers lacking the engineered tag and enabling the assembly of macromolecular structures without potential interferences.

SUMMARY OF THE INVENTION

As a solution to the above-related deficiencies in the prior art, the present invention contemplates using organic acids to purify recombinant spider silks or other non-recombinant structural proteins from *E. coli* bacteria while removing the unwanted bacterial proteins. The invention is based on the unique solubilization and stability characteristics of these proteins, which are resistant to acid hydrolysis for prolonged periods of time at room temperature, while many globular proteins are not. Purified protein solutions can be processed into a spinnable aqueous-based mixture for the production of fibers. The present invention also contemplates an aqueous protein spinning method that closely mimics the natural spinning process of the spider and has the potential to produce fibers with properties that may resemble or improve upon those of natural silk fibers. The present invention represents the first known example of an aqueous process for the spinning of silk proteins into fibers. Furthermore, this invention is the only known report, to date, of spinning recombinant silk proteins into fibers. The present invention displays numerous advantages over the background art, including a purification method with organic acids containing fewer steps, requiring less time and smaller volumes of reagents. The present invention also results in better recovery of protein at a higher purity. For example, the (SP1)₇ protein can be recovered at a level of 150 mg/L, compared to the 7mg/L recovery rate by the current art (*see Prince et al., supra*). While not limited to any mechanism by which a recovery is achieved, it is believed that lower protein recovery rates by the traditional methods are caused, in part, from incomplete binding of the protein to the affinity resin. Such traditional techniques include, but are not limited to, ion exchange chromatography and affinity chromatography. The inability of these proteins to bind to the resin is most likely due to a high degree of secondary structure even in the presence of high concentrations of denaturant. Sample purity from the present invention has been obtained in the range of 94-97% as determined by amino acid analysis (*see Examples 1 and 2, infra*). The current art results in a wide and inconsistent range of purity ranging from 70% (*Prince et al., supra*), to 99% (*Lewis et al., 1996, supra*). While high sample purity is possible using current art by affinity chromatography, the presence of the histidine affinity tag significantly increases the antigenicity of the protein and adversely affects the properties

of fibers, films, or other materials by disrupting the proper molecular orientation required within the material. Also, in many cases the current art results in samples still contaminated by other bacterial proteins, requiring additional purification such as HPLC (*Prince et al.*; *Lewis et al.*, *supra*). Finally, the methods of the present invention are easily scaled-up, and fibers are spun in an environmentally benign solution reducing hazardous waste accumulation and cost. For example, the present invention contemplates the spinning of silk proteins in an environmentally innocuous aqueous based system. In one embodiment of the present invention, a solution of an organic acid is used to effect the lysis of bacteria and initiate purification of recombinant silks and native structural proteins. Globular proteins are hydrolyzed while the silk protein remains intact. Silk proteins remain and are concentrated into an aqueous-based mixture for fiber spinning. The embodiment may comprise the following steps: a) resuspension of the cell pellet in concentrated organic acid and dilution to 2.3N in water (+/-denaturant and/or surfactant) to form a homogeneous mixture; b) incubation at room temperature 1 hour with stirring and centrifugation to remove cell debris; c) reduction of volume, 10-100 fold by ultrafiltration and removal of insoluble material by centrifugation; d) dialysis and removal of insoluble material by centrifugation; e) purification by ion exchange chromatography and dialysis into processing buffer; f) concentration of solution to 11-40% (w/w) protein by ultrafiltration and spinning solution into fibers. While this embodiment is given for guidance, those of skill in the art may choose to add or delete certain steps while remaining within the spirit and scope of the present invention. For example, the purification methodology may be employed with or without the spinning of the fiber solution. Several native and recombinant structural proteins have been purified by this method. Any biological sample containing a structural protein of interest, native or recombinant, is amenable to the methodology outlined in the invention. Examples of biological samples may include, but are not limited to, *E. coli* cells, other bacterial cells, eukaryotic cells, a medium in which a structural protein has been secreted, bone, tissues or organs. And while many variables have been examined and optimized throughout the process, each variable and optimization exemplify variations of the overall general method. Choosing among the various parameters is highly dependent on the protein being prepared. Table 1 below lends guidance to those of skill in the art.

TABLE 1

Variables Explored	Conclusion
Lysis	
1. Type of acid	Protein: (4+1) ₄ Acid: Propionic Protein: (Sp1) ₇ Acid: Formic Protein: NcDS Acid: Formic Protein: OmpF Acid: Valeric Protein: Recognin Acid: Valeric
2. Volume acid/g cells (2-100 ml/g)	Increased acid volume generally decreases purity
3. Acid strength (0.5-23N)	Full strength is best (23N Formic, 13N Propionic) for lysis.
4. Length of lysis (30 min-overnight)	1 hr is preferred
5. Temperature of lysis (25°C-37°C)	No effect
6. Lysis under denaturing conditions	Solubility improves, purity decreases
7. Lysis in the presence of detergents	No effect on purity
Purification	
1. Lysate purification by chromatography	Chromatography successfully purifies target (affinity, ion exchange).
Processing	
1. Urea concentration in the processing buffer (160 mM vs 1M)	1M urea improves solubility slightly
2. Ionic strength of the processing buffer (20-100 mM NaCl)	Increasing NaCl concentration causes precipitation
Spinning	
1. Spin aqueous-based mixture protein concentration (11-35%)	Protein dependent
2. Age of aqueous-based mixture (0-5 days)	Spinnability changes as the aqueous-based mixture ages
3. Temperature during aging (4-30°C)	Higher temperatures accelerate changes in the solution behavior (<i>i.e.</i> spinnability and solubility)
4. Coagulation bath (70-90% methanol)	Methanol percentage affects speed of fiber formation, fiber behavior

A variety of embodiments are contemplated. In one embodiment, the present invention contemplates a method, comprising: a) providing: i) a biological sample comprising one or more structural polypeptides; and ii) an acid; b) treating said sample with said acid under conditions such that said one or more polypeptides is recovered in a solution. A variety of structural peptides are contemplated, including but not limited to polypeptides selected from SEQ ID NO.: 2, SEQ ID NO.: 4, SEQ ID NO.: 6, SEQ ID NO.: 8, SEQ ID NO.:9, and SEQ ID NO.: 11. the peptides may be recombinant or native polypeptides.

A variety of acids are contemplated. Organic acids are preferred. In one embodiment, the present invention contemplates one or more organic acids selected from formic, acetic, propionic, butyric, and valeric acids.

It is the goal to produce fibers. Therefore, in one embodiment, the method further comprises the step of manipulating said solution under conditions such that insoluble fibers are produced. Indeed, the present invention specifically contemplates the fibers produced according to the above-described process.

The present invention specifically contemplates methods wherein recombinant structural proteins are manipulated. In one embodiment, the present invention contemplates a method, comprising: a) providing: i) host cells expressing one or more recombinant structural polypeptides, and ii) a solution comprising an organic acid; b) treating said host cells with said solution to create a mixture; c) removing insoluble material from said mixture; and d) recovering said one or more recombinant polypeptides in a solution. Again, a variety of peptides are contemplated. In one embodiment, one or more polypeptides are selected from SEQ ID NO.: 2, SEQ ID NO.: 4, SEQ ID NO.: 6, SEQ ID NO.: 8, SEQ ID NO.:9, and SEQ ID NO.: 11. Again, a variety of acids are contemplated, including but not limited to organic acids selected from formic acid, acetic acid, propionic acid, butyric acid, and valeric acid.

To produce fibers, the method involves manipulation of said recovered one or more recombinant polypeptides in said solution under conditions such that insoluble fibers are produced. The present invention specifically contemplates the fibers themselves produced according to the above-described process.

A variety of host cells are contemplated for recombinant production. Thus, in one embodiment the present invention contemplates a method, comprising: a) providing: I) bacterial cells expressing one or more recombinant structural polypeptides, and ii) a solution comprising an organic acid selected from formic acid, acetic acid, propionic acid, butyric acid, and valeric acid; b) treating said bacterial cells with said solution to create a mixture; c) removing insoluble material from said mixture; and d) recovering said one or more recombinant polypeptides in a solution. As noted above, a variety of peptides are contemplated, including but not limited to one or more polypeptides is selected from SEQ ID NO.: 2, SEQ ID NO.: 4, SEQ ID NO.: 6, SEQ ID NO.: 8, and SEQ ID NO.: 11.

To produce fibers, said recovered one or more recombinant polypeptides are manipulated under conditions such that insoluble fibers are produced. In a preferred embodiment, said manipulating comprises: a) concentrating said recovered one or more recombinant silk polypeptides to create a concentrated solution; and b) forcing said concentrated solution through a spinneret. The present invention specifically contemplated the fibers themselves which are produced according to this process.

In sum, the present invention contemplates a method, which comprises providing a biological sample composed of a polypeptide and an acid, and manipulating the biological sample under conditions such that the polypeptide is substantially purified into an aqueous-based mixture.

The method, in several embodiments, includes using polypeptides that may be selected from SEQ ID NO.: 2, SEQ ID NO.: 4, SEQ ID NO.: 6, SEQ ID NO.: 8, SEQ ID NO.: 9, and SEQ ID No.: 11 herein, although other amino acid sequences are also contemplated.

In another embodiment of the present invention, the biological sample comprises many types of polypeptides, including, but not limited to, recombinant and non-recombinant polypeptides. Structural polypeptides, such as silk polypeptides, are also contemplated.

In further embodiments of the present invention, organic acids are used to manipulate aqueous-based mixtures under conditions such that the mixtures may be processed into fibers. The organic acids that may be used include, but are not limited to,

formic, acetic, propionic, butyric, and valeric acids. The present invention further contemplates the product that is achieved by the methods that are described herein.

While a variety of applications for the methods and products herein described are contemplated, the applications are not limited. For example, the compositions of the present invention may comprise any type of replacement for, or blended with, high strength light-weight synthetic polymers (e.g., kevlar[®]) for applications such as manufacture of skis, skateboards, and tennis rackets. The method of the present invention can also be used to create a product that can be used as a precursor to the construction of many materials, including, but not limited to, films, fibers, woven articles (e.g., clothing), sutures, ballistic protection, parachutes and parachute cords.

DESCRIPTION OF THE DRAWINGS

To facilitate an understanding of the invention, a number of Figures are included herein.

Figure 1 presents the nucleic acid sequence of a recombinant silk protein (SEQ ID NO: 1) designated $pQE(sp1)_7$.

Figure 2 presents a recombinant silk protein (SEQ ID NO: 2), designated $pQE(SP1)_7$, that is the gene product of the nucleic acid sequence presented in (SEQ ID NO: 1).

Figure 3 presents the nucleic acid sequence of a recombinant silk protein (SEQ ID NO: 3) designated $pQE[(SP1)_4 / (SP2)_1]_4$.

Figure 4 presents a recombinant silk protein (SEQ ID NO: 4), designated $pQE[(SP1)_4 / (SP2)_1]_4$, that is the gene product of the nucleic acid sequence presented in (SEQ ID NO: 3).

Figure 5 presents the nucleic acid sequence of a recombinant silk protein (SEQ ID NO: 5) designated $pET[(SP1)_4 / (SP2)_1]_4$.

Figure 6 presents a recombinant silk protein (SEQ ID NO: 6), designated $pET[(SP1)_4 / (SP2)_1]_4$ that is the gene product of the nucleic acid sequence presented in (SEQ ID NO: 5).

Figure 7 presents the nucleic acid sequence of a recombinant silk protein (SEQ ID NO: 7) designated $pETNcDS$.

Figure 8 presents a recombinant silk protein (SEQ ID NO: 8), designated pETNcDS, that is the gene product of the nucleic acid sequence presented in (SEQ ID NO: 7).

Figure 9 presents a bacterial membrane protein (SEQ ID NO: 9), designated ompF.

Figure 10 presents the nucleic acid sequence of a recombinant structural protein. (SEQ ID NO: 10) designated *Recognin B1*.

Figure 11 presents a recombinant structural protein (SEQ ID NO: 11), designated Recognin B1, that is the gene product of the nucleic acid sequence presented in (SEQ ID NO: 10).

Figure 12 presents a polyacrylamide gel comparing acid lysis purification of the recombinant silk protein pQE(Sp1)₇ to traditional denaturing method. The pQE(Sp1)₇ protein is enriched by acid lysis compared to lysis under denaturing conditions (e.g. 8M urea). Subsequent affinity chromatography purification by Ni-NTA of the formic acid lysate results in a yield comparable to the purification of the traditional denaturing lysate.

Figure 13 represents a polyacrylamide gel depicting the QAE-Sephadex purification scheme with a propionic acid extracted pET[(Sp1)₄/(Sp2)₁]₄ protein sample.

Figure 14 presents a polyacrylamide gel depicting the purification of pET[(Sp1)₄/(Sp2)₁]₄ by lysis with propionic acid with 3M guanidine-HCl and ion-exchange chromatography using QAE-Sephadex A50.

Figure 15 represents a polyacrylamide gel depicting the purification of ompF, a native *E. coli* structural protein from a lyophilized *E. coli* cell pellet. The cell pellet was extracted using valeric acid. This extraction procedure yielded a purity of approximately 85% based on coomassie-blue staining.

Figure 16 presents a polyacrylamide gel of Recognin B1, a recombinant coiled coil structural protein. A cell pellet was lysed in either gel loading buffer, formic acid or valeric acid. Relative amounts of the cell pellet loaded onto the gel were 85, 400, 900 ug for the loading buffer, formic and valeric acid lysates, respectively. Acetic, propionic or butyric acids were unable to extract this protein.

Figure 17 presents photomicrographs of a pETNcDS fiber spun from a protein solution of 25% (w/v) as determined by extinction coefficient. Fibers were generated at a

rate of at 10 ul/min in a 90% methanol coagulation bath. Consistent diameters of about 60 um were observed. Under polarizing light, the color changed uniformly from blue to yellow as the angle of light was changed indicating directional orientation in the fiber.

Figure 18 presents photomicrographs of a fiber spun at a rate of 5 ul/min into a 90 % methanol coagulation bath from a 12.5% aqueous solution of pQE[(Sp1)₄/(Sp2)₁]₄ viewed under a) white light and b) polarized light with a tint plate. The fibers present a consistent diameter of about 30 um.

DEFINITIONS

To facilitate an understanding of the invention, a number of terms are defined.

The term "aqueous", as defined herein, refers to a water miscible solution.

The term "aqueous-based mixture", as defined herein, refers to a protein in an aqueous solution. The mixture may be used for protein purification, fiber spinning, film formation or other materials.

The term "aqueous fiber spinning" refers to a process by which fibers are formed from an aqueous solution.

The terms "spin" "spinnable" as used herein, refers to a mixture that is capable of forming a fiber and the fiber remains intact during manipulation (*i.e.* drawing and removal from a coagulation bath).

The term "biological sample", as defined herein, refers to any sample containing a structural protein of interest, native or recombinant, that is amenable to the methodology of the present invention. Examples of biological samples may include, but are not limited to, *E. coli* cells, other bacterial cells, eukaryotic cells, a medium where the structural protein has been secreted, bone, tissues or organs.

The term "recombinant protein", as used herein, refers to the product produced by expression of a recombinant DNA sequence in a foreign host. The (Sp1)₇ protein, described herein in Example 1, exemplifies a recombinant protein.

The term "recombinant" or "recombining" refers to a nucleic acid sequence which is incorporated into a vector, *e.g.*, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA or a genomic DNA fragment produced by PCR or restriction

endonuclease treatment) independent of other DNA sequences. This definition also includes recombinant DNA which is part of a hybrid gene encoding additional amino acid sequences.

The term "recombinant DNA vector" as used herein refers to DNA sequences containing a desired coding sequence and appropriate DNA sequences necessary for the expression of the operably linked coding sequence in a particular host organism (*e.g.*, bacteria). DNA sequences necessary for expression in prokaryotes include a promoter, optionally an operator sequence, a ribosome binding site and possibly other sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals and enhancers.

The term "non-recombinant" refers to proteins that are derived by other than recombinant means. Non-recombinant protein may be structural or non-structural. The *E. coli* OmpF membrane protein (described herein in Example 6), which is, in this case, a naturally occurring protein that serves as an example of a non-recombinant protein.

The term "lyophilized pellet" represents a sample that is derived from a biological sample where the sample is frozen and dried under vacuum (-50°C & 10-100 microns of Hg) to produce a powder.

The term "purified" or a "pure preparation" of a polypeptide, as used herein, means a polypeptide that has been separated from other proteins, lipids, and nucleic acids with which it naturally occurs. The polypeptide is also separated from substances, *e.g.*, antibodies or gel matrix, *e.g.*, polyacrylamide, which are used to purify it. The term "substantially purified" polypeptide of the present invention constitutes at least 50%, and often above 90%, of the purified preparation as based on amino acid analysis.

The term "acid" for the purposes of the present invention, refers to any organic acid that is capable of hydrolyzing contaminating proteins while allowing silk or other structural proteins to remain intact. Formic, acetic, propionic, butyric, and valeric acids are all examples of organic acids, although other acids are also contemplated.

For the purposes of this invention, we define a "protein" as a polymer in which the monomers are amino acids and which are joined together through amide bonds and alternatively referred to as a polypeptide. The terms "protein" and "polypeptide" are herein used interchangeably. Standard abbreviations for amino acids are used (*e.g.*, P for proline). These abbreviations are included in *Stryer, Biochemistry, Third Ed., (1988)*,

which is incorporated herein by reference for all purposes.

The term "silk polypeptide" refers to a protein that approximates the molecular and structural profile of native silk proteins and fibers.

5 The term "structural protein" or "structural polypeptide" refers to a class of non-catalytic proteins that may serve as a biological structural support. The proteins may serve as biological structural supports by themselves, in conjunction with other proteins, or as a matrix or support for other materials. Examples from this class include, but are not limited to, proteins such as spider silks, that are used for spider web architecture; porin proteins, which form channels in biological membranes; keratin, the major
10 structural component of hair; collagen, the major extracellular protein in connective tissue. The silks, OmpF and recognin proteins described herein are examples of structural proteins.

The term "recovered" refers to the process by which protein is locally sequestered and captured.

15 The term "organic acid" refers to the class of acids, such as formic, acetic, propionic, butyric, and valeric acids, which are found in living organisms but not necessarily, derived from said living organism. Said organic acids can also be obtained from commercial vendors (e.g. Sigma Chemical).

As used herein, the term "host cell" refers to any cell capable of expressing a
20 functional gene and/or gene product introduced from another cell or organism. This definition includes *E. coli.*, as well as other organisms.

The term "insoluble fibers" refers to proteinaceous fibers that will not solubilize in an aqueous solution.

25 The term "bacterial" refers to any of numerous groups of microscopic, one-celled organisms including, but not limited to the phylum Eubacteria of the kingdom Procaryotae.

The term "concentrating" refers to any process that increases the molarity of proteinaceous solution.

30 The term "concentrated solution" refers to a proteinaceous solution adjusted to a predetermined molarity higher than said pre-adjusted proteinaceous solution.

The term "spinneret" refers to a small orifice used for fiber formation.

DESCRIPTION OF THE INVENTION

A number of different embodiments, as exemplified in the examples, of the present invention are contemplated, including the scaling-up of the method, automation of the method, or use of the method to purify other structural proteins.

One of skill in the art will recognize that the practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, microbiology, and recombinant DNA, which are within the skill of the art. Such techniques are described in the literature. See, for example, *Molecular Cloning, A Laboratory Manual*, 2nd Ed., by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); *DNA Cloning*, Volumes I and II (D. N. Glover ed., 1985); *Oligonucleotide Synthesis* (M. J. Gait ed., 1984); *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu et al. eds.).

The proteins of the present invention can be made by direct synthesis (chemically or biologically) or by expression from cloned DNA. The source of the protein is not limited to recombinant means. Non-recombinant proteins may be purified or spun using the methods described herein. Indeed, Example 6, *infra*, describes the purification of *E. coli* OmpF membrane protein, which is, in this case, a naturally occurring (i.e. non-recombinant protein) protein.

The means for expressing cloned DNA are generally known in the art. However, there are some considerations for design of expression vectors that are unusual for expressing DNA encoding the spider silk proteins of the present invention. For example, the proteins are highly repetitive in their structure. Accordingly, cloned DNA should be propagated and expressed in host cell strains that will maintain repetitive sequences in

extrachromosomal elements (e.g. SURETM cells, Stratagene). Also, due to the high content of alanine, glycine, proline, and glutamine, it might be advantageous to use a host cell which over expresses tRNA for these amino acids.

The present invention contemplates the use of many different organic acids to manipulate recombinant and non-recombinant biological samples under conditions such that a polypeptide is substantially purified. While the use of *E. coli* cells with formic, propionic and valeric acid are contemplated, the present invention is not limited to these particular embodiments, but may also be practiced using other organic acids, such as acetic, and butyric, acids, all of which serve as examples. The present invention may also be practiced using other prokaryotic or eukaryotic cells (aside from, or along with, *E. coli* cells), the media in which the protein-of-interest has been secreted, organs, tissue, bone and other components, all of which are examples of biological sample materials.

EXPERIMENTAL

The following examples serve to illustrate certain embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

EXAMPLE 1

Purification of Recombinant Silk Protein

with Formic Acid and Ion Exchange Chromatography

In this example, the gene product of *pQE(sp1)₇* (SEQ ID NO: 1), as set out in Figure 1, is expressed as recombinant silk protein *pQE(SP1)₇* (SEQ ID NO: 2), as set out in Figure 2, in *E. coli* as described elsewhere (*Prince et al.*, 1995). The *(sp1)₇* gene was cloned into the expression vector *pQE-9* (Qiagen) and transformed into the host cell line SG13009pREP4 (Stratagene). Cultures were grown to an $A_{600} = 1.5-2.0$ in 4xYT medium (per liter: 32 g tryptone, 20 g yeast extract, 5 g NaCl) containing 400ug/mL ampicillin. Protein expression was induced by the addition of isopropyl-thiogalactopyranoside (IPTG) to a final concentration of 1mM. After 1-4 hours the cells were harvested by centrifugation and stored for purification. Lyophilized pellets were lysed in 23N formic acid (100 ml/g cell pellet), diluted to 4.6N acid with distilled and deionized water and stirred 1 hour at room temperature. The cell lysate was clarified by

centrifugation and concentrated 20 fold by ultrafiltration. The solution was clarified by centrifugation and the supernatant was dialyzed extensively into 8M urea, 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH 8. Precipitated material was removed by centrifugation and the clarified supernatant was applied to an affinity chromatography resin (nickel-NTA agarose) that had been equilibrated with 8M urea, 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH 8. The chromatography conditions were designed to bind the recombinant silk protein, but let the remaining bacterial proteins pass through the column. The column was washed with 8M urea, 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH 7. The pQE(SP1)₇ protein was eluted from the column 8M urea, 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH 3. The sample was 94% pure as determined by quantitative amino acid analysis. Figure 12 illustrates a comparison of traditional purification techniques with the methodology enclosed in this application. Cells lysed with formic acid yielded more silk protein with a similar purity when compared to the 6M guanidine lysis with Ni-NTA affinity chromatography.

EXAMPLE 2

Purification of Recombinant Silk Protein with Propionic Acid and Ion Exchange Chromatography

In this example, the gene product of $pQE[(SP1)_4 / (SP2)_1]_4$ (SEQ ID NO: 3), as set out in Figure 3, is expressed as recombinant silk protein pQE[(SP1)₄ / (SP2)₁]₄ (SEQ ID NO: 4), as set out in Figure 4, in *E. coli* (Prince et al., 1995). The $[(SP1)_4 / (SP2)_1]_4$ gene was cloned into the expression vector pQE-9 (Qiagen) and transformed into the host cell SG13009pREP4 (Stratagene). Cultures were grown to an $A_{600} = 1.5-2.0$ in 4xYT medium (per liter: 32 g tryptone, 20 g yeast extract, 5 g NaCl) containing 400ug/mL ampicillin. Protein expression was induced by the addition of IPTG to a final concentration of 1mM. After 1-4 hours the cells were harvested by centrifugation and stored for purification. Lyophilized pellets were lysed in 13.3N propionic acid (2 ml/g cell pellet), diluted to 2.3N acid with distilled and deionized water and stirred 1 hour at room temperature. The cell lysate was clarified by centrifugation and concentrated 20 fold by ultrafiltration. Many of the acid stable proteins became insoluble and were

removed by centrifugation. The clarified supernatant was dialyzed extensively into 10 mM Tris, pH 9 containing 2M urea. The dialyzed solution was applied to a strong anion exchange resin, QAE-Sephadex A50, that had been equilibrated with 10 mM Tris, pH 9 containing 2M urea. The chromatography conditions were designed such that the positively charged silk protein would not bind to the column, but the remaining proteins with lower isoelectric points and net negative charge would bind to the column. The column was washed with 10 mM Tris, pH 9 containing 2M urea to recover any remaining silk protein. The wash was pooled with the unbound silk containing fraction and processed. The sample was 97% pure as determined by quantitative amino acid analysis.

EXAMPLE 3

Purification of Recombinant Silk Protein with Propionic Acid and Ion Exchange Chromatography

In this example, the gene product of *pET [(SP1)₄ / (SP2)₁]₄* (SEQ ID NO: 5), as set out in Figure 5, is expressed as recombinant silk protein pET[(SP1)₄/(SP2)₁]₄ (SEQ ID NO: 6), as set out in Figure 6, in *E.coli* (Prince *et al.*, 1995). The [(SP1)₄/(SP2)₁]₄ gene was cloned into the expression vector pET24 (Novagen Inc.) and transformed into the host cell BL21(DE3) pLysS. Cultures were grown to an A₆₀₀ = 19 in defined medium (per liter: 13.3 g KH₂PO₄, 4 g (NH₄)₂HPO₄, 1.7 g Citric acid, 25 g glucose, 1.2 g MgSO₄·7H₂O, 39 mg FeCl₃, 13 mg MnSO₄·H₂O, 10 mg ZnSO₄·7H₂O, 3 mg H₃BO₃, 2.5 mg Na₂MoO₄·2 H₂O, 2.5 mg CoCl₂·6H₂O, 1.8 mg Cu(CH₃COO)₂·H₂O, 6.7 mg EDTA, 4.5 mg thiamine-HCl) with kanamycin (30 ug/ml) at 37°C, 16 liter/min air and 600 rpm. Expression was induced for 1 hr with 1 mM IPTG at which time the cells were harvested by centrifugation and stored for purification. Lyophilized pellets were lysed in 13.3N propionic acid (2 ml/g cell pellet), diluted to 2.3N acid with distilled and deionized water and stirred 1 hour at room temperature. The cell lysate was clarified by centrifugation and concentrated 20 fold by ultrafiltration. Many of the acid stable proteins became insoluble and were removed by centrifugation. The clarified supernatant was dialyzed extensively into 10 mM Tris, pH 9 containing 2M urea. The dialyzed solution was applied to a strong anion exchange resin QAE-Sephadex A50 that had been equilibrated with 10 mM Tris, pH 9 containing 2M urea. The chromatography conditions were

designed such that the positively charged silk protein would not bind to the column, but the remaining proteins with lower isoelectric points and net negative charge would bind to the column. The column was washed with 10 mM Tris, pH 9 containing 2M urea to recover any remaining silk protein. The wash was pooled with the unbound silk containing fraction and processed. The sample was 75-85% pure as determined by coomassie-blue staining of a polyacrylamide gel (see Figure 13).

EXAMPLE 4

Purification of Recombinant Silk protein with Propionic Acid Containing Denaturant and Ion Exchange Chromatography

In this example, the gene product of *pET[(SP1)₄ / (SP2)₁]₄* (SEQ ID NO: 5), as set out in Figure 5, is expressed as recombinant silk protein pET[(SP1)₄ / (SP2)₁]₄ (SEQ ID NO: 6), as set out in Figure 6, in *E.coli* (Prince et al., 1995). Lyophilized pellets were lysed in 13.3N propionic acid (2 mL/g cell pellet), diluted to 2.3N acid with 6M guanidine hydrochloride (to a final concentration of 3M) and distilled and deionized water and stirred for 1 hour at room temperature. The cell lysate was clarified by centrifugation and concentrated 3 fold by ultrafiltration. Precipitated material was removed by centrifugation and the clarified supernatant was dialyzed extensively into 10 mM Tris, pH 9 containing 2M urea. Many of the acid stable proteins became insoluble and were removed by centrifugation. The dialyzed supernatant was applied to a strong anion exchange resin, QAE-Sephadex A50 that had been equilibrated with 10 mM Tris, pH 9 containing 2M urea. The chromatography conditions were designed such that the positively charged silk protein would not bind to the column, but the remaining proteins with lower isoelectric points and net negative charge would bind to the column. The column was washed with 10 mM Tris, pH 9 containing 2M urea to recover any remaining silk protein (see Figure 14). The wash was pooled with the unbound silk containing fraction and processed as describe in example 9. This sample was approximately 80% pure based on coomassie blue staining.

EXAMPLE 5

Purification of Recombinant Silk Protein with Formic Acid Containing Denaturant and Affinity Chromatography

In this example, the gene product of *pETNcDS* (SEQ ID NO: 7), as set out in Figure 7, is expressed as recombinant silk protein pETNcDS (SEQ ID NO: 8), as set out in Figure 8, in *E. coli* (Arcidiacono *et al.* 1998). The NcDS gene was cloned into the expression vector pET24 (Novagen Inc.) and transformed into the host cell BL21(DE3) pLysS. Cultures were grown to an $A_{600} = 4$ in 4xYT medium (per liter: 32 g tryptone, 20 g yeast extract, 5 g NaCl) with kanamycin (30 ug/ml) at 37°C, 1 liter/min air and 800 rpm. Expression was induced for 3 hr with 1 mM IPTG at which time the cells were harvested by centrifugation and stored for purification. Lyophilized pellets were lysed in 23N formic acid (5 ml/g cell pellet), diluted to 2.3N acid with 6M guanidine hydrochloride (to a final concentration of 3M) and distilled and deionized water and stirred 1 hour at room temperature. The cell lysate was clarified by centrifugation and concentrated 20 fold by ultrafiltration. The solution was clarified by centrifugation and the supernatant was dialyzed extensively into 8M urea, 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH 8. Precipitated material was removed by centrifugation and the clarified supernatant was applied to an affinity chromatography resin (nickel-NTA agarose) that had been equilibrated with 8M urea, 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH 8. The chromatography conditions were designed to bind the recombinant silk protein, but let the remaining bacterial proteins pass through the column. The column was washed with 8M urea, 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH 7. The NcDS protein was eluted from the column 8M urea, 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH 3. The purified protein could then be processed for fiber spinning as in Example 8.

EXAMPLE 6

Valeric Acid Purification of *E. coli* OmpF Membrane Protein

In this example, a native *E. coli* ompF membrane protein (SEQ ID NO: 9), as presented in Figure 9, was purified. Cells were grown and harvested as described in Example 3. Because OmpF is a native *E. coli* protein, its production was not induced by the addition of IPTG. Lyophilized pellets were lysed in 9.2N valeric acid (2 mL/g of

pellet), diluted to 2.3N acid with distilled and deionized water and stirred for 1 hour at room temperature. The cell lysate was clarified by centrifugation and applied to an SDS polyacrylamide gel for electrophoresis. Figure 15 represents the polyacrylamide gel depicting this purification of ompF, a native *E. coli* structural protein from a lyophilized *E. coli* cell pellet. The ompF protein was then blotted onto a nitrocellulose membrane for N-terminal sequencing. The resulting 30 amino acids of N-terminal sequence led to the identification of *E. coli* outer membrane protein, ompF. This simple extraction procedure yielded a purity of approximately 85% based on coomassie-blue staining.

EXAMPLE 7

Organic Acid Extraction of Recognin B1 Protein

In this example, the gene product of *Recognin B1* (SEQ ID NO: 10), as set out in Figure 10, was expressed as recombinant synthetic coiled protein Recognin B1 (SEQ ID NO: 11), as set out in Figure 11, in *E. coli* (McGrath, K.P. and Kaplan, D.L. *Mat. Res. Symp. Proc.* 292, 83-91). The *Recognin B1* gene was cloned into the expression vector pQE-9 (Qiagen) and transformed into the *E. coli* host cell, SG13009pREP4 (Qiagen). Cultures were grown to an A₆₀₀ of 1 in 4xYt medium (per liter: 32 g tryptone, 20 g yeast extract, 5 g NaCl) with ampicillin (400 ug/mL) and kanamycin (50 ug/mL). Expression was induced for two hours with 1 mM IPTG at which time the cells were harvested by centrifugation and stored for purification. Individual lyophilized pellets were lysed separately in 23N formic acid, 17.5N acetic acid, 13.4N propionic acid, 10.9N butyric acid or 9.2N valeric acid (2 mL/g of pellet), diluted to 2.3N acid with distilled and deionized water and stirred for 1 hour at room temperature. The cell lysates were clarified by centrifugation and analyzed by SDS-PAGE. Figure 16 presents the polyacrylamide gel of Recognin B1, a recombinant coiled coil structural protein. A cell pellet was lysed in either gel loading buffer, formic acid or valeric acid. Relative amounts of the cell pellet loaded onto the gel were 85, 400, 900 ug for the loading buffer, formic and valeric acid lysates, respectively. Acetic, propionic or butyric acids were unable to extract this protein. The results indicated that formic and valeric acids were able to extract a significant quantity of Recognin B1 from *E. coli* pellets. The extracted protein did not appear to be degraded upon exposure to these organic acids. Of the two

acids, valeric acid was able to extract Recognin B1 in a relatively pure form.

EXAMPLE 8

Processing and Fiber Spinning of Recombinant Silk Protein

5 Recombinant pETNcDS protein was purified as in Example 5, concentrated 100-fold by ultrafiltration and dialyzed into 10 mM NaH_2PO_4 , 1mM Tris, 20 mM NaCl, pH5 containing 1M urea. The dialyzed sample was clarified by centrifugation and concentrated by ultrafiltration to a 25% (w/w) solution for fiber spinning. A Harvard Apparatus Infusion/Withdrawal Pump (Harvard Instruments, Natick MA) was used with
10 a specialized microspinner (cavity volume 0.5 ml), and a 6 cm (0.005 I.D.) piece of tubing which was used as a spinneret. The silk solution was forced through the spinneret at a rate of 5-10 ul/min into a coagulation bath consisting of 90% methanol. Water insoluble fibers, 10-60 um in diameter, were produced and prepared for light microscopy (see Figure 17).

EXAMPLE 9

Processing and Fiber Spinning the pET[(Sp1)₄/(Sp2)₁]₄ Recombinant Silk Protein

pET[(SP1)₄/(SP2)₁]₄ (SEQ ID NO: 6) was purified as described in Example 4. The sample was clarified by centrifugation and concentrated by ultrafiltration to 9.3%
20 (w/w) solution for fiber spinning. A Harvard Apparatus Infusion/Withdrawal Pump (Harvard Instruments, Natick MA) was used with a specialized microspinner (cavity volume 0.5 ml) and a 6 cm (0.005 I.D.) piece of tubing which was used as a spinneret. The silk solution was forced through the spinneret at a rate of 2-5 ul/min into a coagulation bath consisting of 90% methanol. Fibers were produced from the solution.
25 Fibers from the 9.3% solution were removed from the coagulation bath: said fibers were water insoluble and were subsequently prepared for light microscopy.

EXAMPLE 10

Processing and Fiber Spinning the pQE[(Sp1)₄/(Sp2)₁]₄ Recombinant Silk Protein

The pQE[(Sp1)₄/(Sp2)₁]₄ protein was purified by lysis in formic acid/guanidine hydrochloride as in Example 5 and dialyzed into 10 mM NaH₂PO₄, 1 mM Tris, 20 mM NaCl, pH 5 containing 160 mM urea. The dialyzed sample was clarified by centrifugation and concentrated by ultrafiltration to 6.5% and 12.5% (w/w) solution for fiber spinning. A Harvard Apparatus Infusion/Withdrawal Pump (Harvard Instruments, Natick MA) was used with a specialized microspinner (cavity volume 0.5 ml) and a 6 cm (0.005" I.D.) piece of tubing was used as a spinneret. The silk solution was forced through the spinneret at a rate of 5-10 ul/min into a coagulation bath consisting of 90% methanol. Fibers were produced from each solution. Only fibers from the 12.5% solution could be removed from the coagulation bath; they were water insoluble and prepared for light microscopy (see Figure 18).

From the above description and examples, it should be clear that the present invention provides improved methods for purifying structural proteins and spinning spider silk proteins. Accordingly, this invention is not limited to the particular embodiments disclosed, but is intended to cover all modifications that are within the spirit and scope of the invention as defined by the appended claims.

CLAIMS

We Claim:

1. A method, comprising:

- 5 a) providing: i) a biological sample comprising one or more structural polypeptides; and ii) an acid;
- b) treating said sample with said acid under conditions such that said one or more polypeptides is recovered in a solution.

10 2. The method of Claim 1, wherein said polypeptide is selected from SEQ ID NO.: 2, SEQ ID NO.: 4, SEQ ID NO.: 6, SEQ ID NO.: 8, SEQ ID NO.:9, and SEQ ID NO.: 11.

15 3. The method of Claim 1, wherein said biological sample comprises recombinant polypeptides.

 4. The method of Claim 1, wherein said biological sample comprises non-recombinant polypeptides.

20 5. The method of Claim 1, wherein said acid comprises an organic acid.

 6. The method of Claim 5, wherein said organic acid is selected from formic, acetic, propionic, butyric, and valeric acids.

25 7. The method of Claim 1, further comprising the step of manipulating said solution under conditions such that insoluble fibers are produced.

 8. The fibers produced according to the process of Claim 7.

30 9. A method, comprising:

- a) providing: i) host cells expressing one or more recombinant structural polypeptides, and ii) a solution comprising an organic acid;

- b) treating said host cells with said solution to create a mixture;
- c) removing insoluble material from said mixture; and
- d) recovering said one or more recombinant polypeptides in a solution..

5 10. The method of Claim 9, wherein said one or more polypeptides is selected from SEQ ID NO.: 2, SEQ ID NO.: 4, SEQ ID NO.: 6, SEQ ID NO.: 8, SEQ ID NO.: 9, and SEQ ID NO.: 11.

10 11. The method of Claim 9, wherein said organic acid is selected from formic acid, acetic acid, propionic acid, butyric acid, and valeric acid.

15 12. The method of Claim 9, wherein said recovered one or more recombinant polypeptides in said solution are manipulated under conditions such that insoluble fibers are produced.

15 13. The fibers produced according to the process of Claim 12.

15 14. A method, comprising:

- a) providing: i) bacterial cells expressing one or more recombinant structural polypeptides, and ii) a solution comprising an organic acid selected from formic acid, acetic acid, propionic acid, butyric acid, and valeric acid.;
- b) treating said bacterial cells with said solution to create a mixture;
- c) removing insoluble material from said mixture; and
- d) recovering said one or more recombinant polypeptides in a solution.

25 15. The method of Claim 14, wherein said one or more polypeptides is selected from SEQ ID NO.: 2, SEQ ID NO.: 4, SEQ ID NO.: 6, SEQ ID NO.: 8, and SEQ ID NO.: 11.

30 16. The method of Claim 14, further comprising the step of manipulating said recovered one or more recombinant polypeptides under conditions such that insoluble

fibers are produced.

17. The method of Claim 16, wherein said manipulating comprises:

- a) concentrating said recovered one or more recombinant polypeptides to create a concentrated solution; and
- b) forcing said concentrated solution through a spinneret.

18. The fibers produced according to the process of Claim 17.

ABSTRACT

Methods are described for the purification and spinning of recombinant and non-recombinant proteins. Specifically, the lysis of bacteria and purification of silk proteins
5 occur in a single solution of organic acid. Bacterial proteins are hydrolyzed while the silk protein remains intact. Silk proteins remain soluble as they are concentrated into a aqueous-based mixture for fiber spinning.

Figure 1: *pQE(Sp1)*, Nucleic Acid Sequence (designated SEQ ID NO. 1)

ATGAGAGGATCGCATCACCATCACCATCACGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGTGGCCAG
GGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGC
CTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCC
GCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGT
AGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGC
CGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGG
TGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCT
GGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGC
GGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAG
AGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGG
CGGTGCCGGCCAAGGYGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGG
CCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGG
CGCCTGGGTTCTCAGGGGACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

ATGAGAGGATCGCATCACCATCACCATCACGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGTGGCCAG
GGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGC
CTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCC
GCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGT
AGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGC
CGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGG
TGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCT
GGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGC
GGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAG
AGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGG
CGGTGCCGGCCAAGGYGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGG
CCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGG
CGCCTGGGTTCTCAGGGGACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

[illegible]

MRGSHHHHHHGSMA¹SGRGGLGGQGAGAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAA
AAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAGGAGQGGYGGLGSQGTSGRGGLG
GQGAGAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAGGAGQGGYGGLGSQ
GTSGRGGLGGQGAGAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAGGAGQ
GGYGGLGSQGTSGIRPAAKLN.

Figure 3. *pQE [(SP1)/(SP2)]₄* Nucleic Acid Sequence (designated SEQ ID NO. 3)

ATGAGAGGATCGCATCACCATCACCATCACGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGTGGCCAG
GGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGC
CTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCC
GCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGT
AGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGC
CGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGG
TGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCT
GGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAACTAGCGGTAGAGGCGGGCT
GGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTG
GCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGG
CTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGG
GACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCC
GCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTG
GGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGG
CTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAACTAGCGG
TAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTG
CCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGG
GTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCC
TGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCG
CGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGT
GAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCC
GGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAA
CAAACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGC
CGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCT
GGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTG
GCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGG
CTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGG
GACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCC
GCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTAT
GGTCCGGGTCAACAACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

Figure 4: pQE [(SP1)₄/(SP2)₁]₄Amino Acid Sequence (designated SEQ ID NO. 4)

MRGSHHHHHHGSMA SGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAA
AAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGRG
GLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGPGGYGPGQQTSGRGGLGGQGAGAAAAAAAAA
AGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAG
AAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGP
GGYGPGQQTSGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAA
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AGAAAAAAAAAAGGAGQGGYGGLGSQGTSGPGGYGPGQQTSGRGGLGGQGAGAAAAAAAAAAGGAG
QGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAA
AAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGPGGYGP
GQQTSGIRRPAAKLN.

Figure 5: *pET [(SP1)/(SP2)]₄* Nucleic Acid Sequence (designated SEQ ID NO. 5)

ATGGCTAGCATGACTGGTGGACAGCAAATGGGTTCGCGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGT
GGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTAT
GGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCG
GCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACT
AGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGG
CGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGG
CCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGG
CGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAAACTAGCGGTAGAGG
CGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCA
AGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGG
TGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTC
TCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAG
CGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGG
GGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCA
GGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAAACT
AGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGG
CGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGG
CCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGG
CGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGC
TGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAG
CGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCG
GTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGG
GTCAACAAACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCA
GCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGC
GGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCA
AGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGG
TGCGGCTGCGGCTGCCGCGGCAGCGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTC
TCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAG
CGGCCGAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCG
GTTATGGTCCGGGTCAACAAACTAGTGGGATCCGAATTCGAGCTCCGTCGACAAGCTTCGAGCACCACC
ACCACCACCACTGA

Figure 6: pET [(SP1)₄/(SP2)₁]₄Amino Acid Sequence (designated SEQ ID NO. 6)

MASMTGGQQMGRGSMASGRGGLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGA
AAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGRG
GLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGPGGYGPGQQTSGRGGLGGQGAGAAAAAAA
GGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGA
AAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGPGGY
GPGQQTSGRGGLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAG
GAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAA
AAAAAAGGAGQGGYGGLGSQGTSGPGGYGPGQQTSGRGGLGGQGAGAAAAAAAAGGAGQGGYGGL
GSQGTSGRGGLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAGG
AGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAGGAGQGGYGGLGSQGTSGPGGYGPGQQTSGIRIR
APSTSFEHHHHHH

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Figure 7: *pETNcDS* Nucleic Acid Sequence (designated SEQ ID NO. 7)

ATGGCTAGCATGACTGGTGGACAGCAAATGGGTTCGGATCCGAATTCGTGGATATGGAGGTCTTGGTGGAC
CAAGGTGCCGGACAAGGAGCTGGTGCAGCCGCCGCAGCAGCAGCTGGTGGTGGCCGGACAAGGAGGATA
TGGAGGTCTTGGAAGCCAAGGTGCTGGACGAGGTGGACAAGGTGCAGGCGCAGCCGCAGCCGCAGCTG
GAGGTGCTGGTCAAGGAGGATACGGAGGTCTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGGTGGA
CAAGGTGCAGGTGCAGCAGCAGCAGCTGGAGGTGTCGGACAAGGAGGACTAGGTGGACAAGGTGCTGG
ACAAGGAGCTGGAGCAGCTGCTGCAGCAGCTGGTGGTGGCCGGACAAGGAGGATATGGAGGTCTCGGAA
GCCAAGGTGCAGGACGAGGTGGATCAGGTGGACAAGGGGCAGGTGCAGCAGCAGCAGCAGCTGGAGGT
GCCGGACAAGGAGGATATGGAGGTCTTGGAAGCCAAGGTGCAGGACGAGGTGGATTAGGTGGACAGGG
TGCAGGTGCAGCAGCAGCAGCAGCAGCCGGAGGTGCTGGACAAGGAGGATACGGTGGTCTTGGTGGAC
AAGGTGCCGGACAAGGTGGCTATGGAGGACTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGGTGGA
CAAGGTGCAGGTGCAGCAGCAGCAGCTGGAGGTGCCGGACAAGGAGGACTAGGTGGACAAGGAGCTGG
AGCAGCCGCTGCAGCAGCTGGTGGTGGCCGGACAAGGAGGATATGGAGGTCTTGGAAGCCAAGGTGCTG
GACGAGGTGGACAAGGTGCAGGCGCAGCCGCAGCAGCAGCCGGAGGTGCTGGACAAGGAGGATACGGT
GGACAAGGTGCCGGACAAGGAGGCTATGGAGGACTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGG
TGGACAAGGTGCAGGTGCAGCAGCAGCAGCAGCAGCTGGAGGTGCCGGACAAGGAGGATTAGGTG
GACAAGGTGCAGGTGCAGCAGCAGCAGCTGGAGGTGCTGGACAAGGAGGATTAGGTGGACAAGGT
GCTGGACAAGGAGCTGGAGCAGCCGCTGCAGCAGCCGCTGCAGCAGCTGGTGGTGTAGACAAGGAGG
ATATGGAGGTCTTGGAAGCCAAGGTGGACGAGGTGGACAAGGTGCAGGCGCAGCCGCAGCAGCAG
CCGGAGGTGCTGGACAAGGAGGATATGGTGGTCTTGGTGGACAAGGTGTTGGACGAGGTGGATTAGGTG
GACAAGGTGCAGGCGCAGCGGCAGCTGTTGGTGGTGGACAAGGAGGATATGGTGGTGTGGTTCTGGGG
CGTCTGCTGCCTCTGCAGCTGCATCCCGTTTGTCTTCTCTCAAGCTAGTTCAAGAGTTTCATCAGCTGTT
TCCAACCTGGTTGCAAGTGGTCCTACTAATTCTGCGGCCTTGTCAAGTACAATCAGTAATGTGGTTTCAC
AAATAGGCGCCAGCAATCCTGGTCTTTCTGGATGTGATGTCCTCATTCAAGCTCTTCTCGAGCACCA
CCACCACCACTGAA

Figure 8: pETNcDS Amino Acid Sequence (designated SEQ ID NO.8)

MASMTGGQQMGRIRIRGYGGLGGQGAGQGAGAAAAAAGGAGQGGYGGLGSQGAGRGGQGAGAAAAA
AGGAGQGGYGGLGSQGAGRGGGLGGQGAGAAAAAGGVGQGLGGQGAGQGAGAAAAAAGGAGQGGYG
GLGSQGAGRGGSGGQGAGAAAAAAGGAGQGGYGGLGSQGAGRGGGLGGQGAGAAAAAAGGAGQGGYG
GLGGQGAGQGGYGGLGSQGAGRGGGLGGQGAGAAAAAAGGAGQGGGLGGQGAGAAAAAAGGAGQGGYGGL
GSQGAGRGGQGAGAAAAAAGGAGQGGYGGQGAGQGGYGGLGSQGAGRGGGLGGQGAGAAAAAAGGA
QGGLGGQGAGAAAAAAGGAGQGGGLGGQGAGQGAGAAAAAAGGVVRQGGYGGLGSQGAGRGGQ
GAGAAAAAAGGAGQGGYGGLGGQGVGAGGLGGQGAGAAAVGAGQGGYGGVGSASAASAAASRLSS
PQASSRVSSAVSNLVASGPTNSAALSSTISNVVSQIGASNPGLSGCDVLIQALLGHHHHHH.

Figure 9: OmpF Amino Acid Sequence, designated SEQ ID NO. 9, without the Signal Sequence (as recovered from *E. coli* by Valeric Acid)

AEIYNKDGKVDLYGKAVGLHYFSKNGENSYGGNGDMTYARLGFKGETQINSDLTGYGQWEY
NFQGNNSGADAQTGNKTRLAFAGLKYADVGSFDYGRNYGVVYDALGYTDMLPEFGGDTAYSD
DFFVGRVGGVATYRNSNFFGLVDGLNFAVQYLGKNERDTARRSNGDGVGGSISYEYEGFGIVGAY
GAADRTNLQEAQPLGNGKKAQWATGLKYDANNIYLAANYGETRNPITNKFTNTSGFANKTQ
DVLLVAQYQFDFGLRPSIAYTKSKAKDVEGIGDVDLVNYFEVGATYYFNKNMSTYVDYIINQIDS
DNKLGVGSDDTVAVGIVYQFA

1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18		19		20		21		22		23		24		25		26		27		28		29		30		31		32		33		34		35		36		37		38		39		40		41		42		43		44		45		46		47		48		49		50		51		52		53		54		55		56		57		58		59		60		61		62		63		64		65		66		67		68		69		70		71		72		73		74		75		76		77		78		79		80		81		82		83		84		85		86		87		88		89		90		91		92		93		94		95		96		97		98		99		100	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100																																																																																																				

ATGAGAGGATCGCATCACCATCACCATCACGGATCCATGGCTAGCGGTGACCTGAAAAACAA
AGTGGCCAGCTGAAAAGGAAAGTTAGATCTCTGAAAGATAAAGCGGCTGAACTGAAACAAG
AAGTCTCGAGACTGAAAAATGAAATCGAAGACCTGAAAGCCAAAATTGGTGACCTGAATAAC
ACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

[illegible][illegible]

Figure 12

Comparative Gel of Acid Lysis vs. Traditional Denaturing Conditions

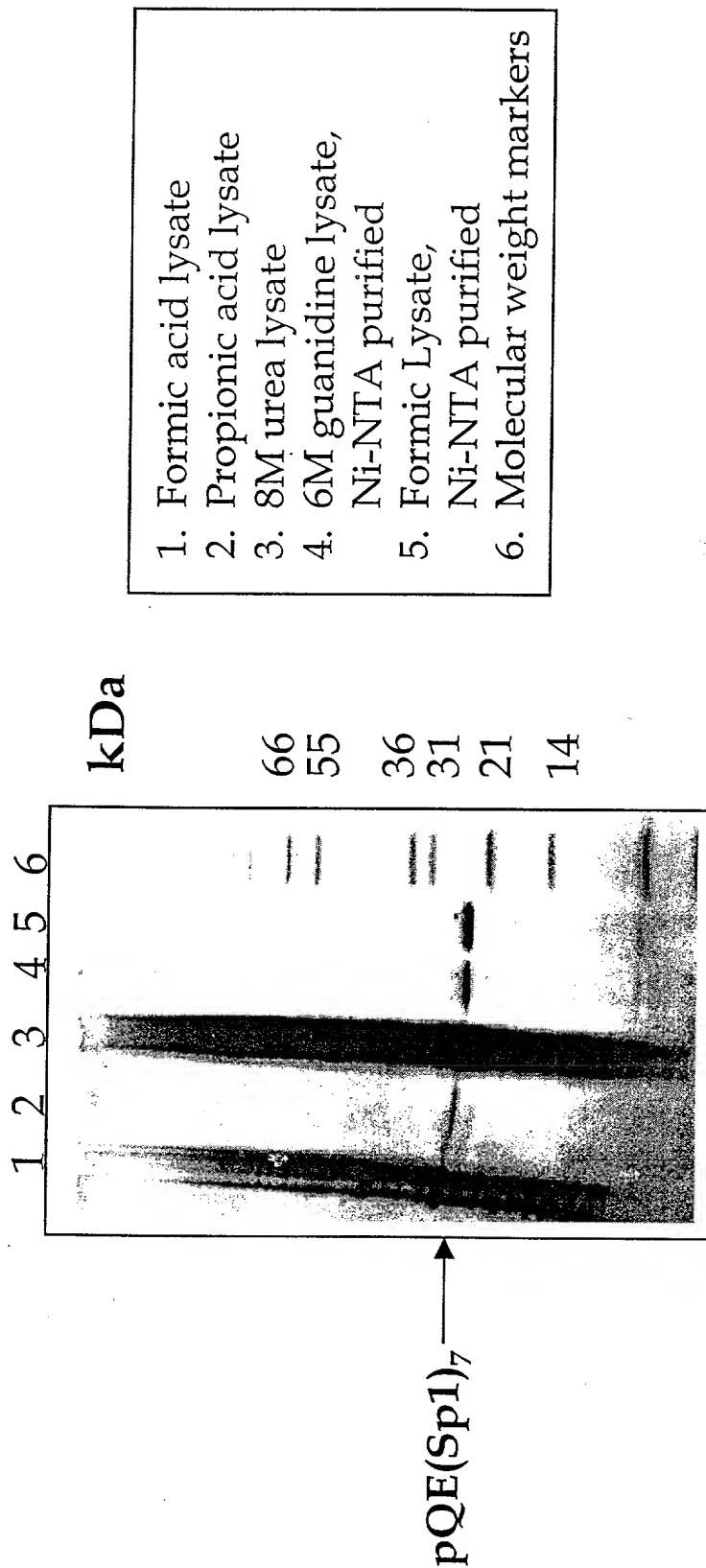
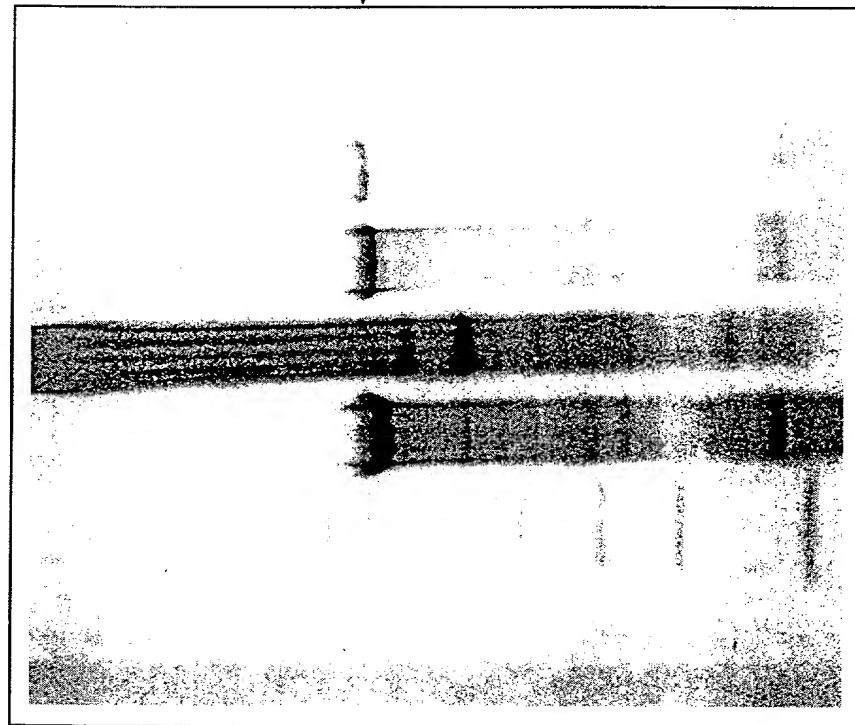


Figure 13

Gel of QAE-Sephadex Purification
of Propionic Acid (PA) Extracted pET[(Sp1)₄/(Sp2)₁]₄ Protein

1 2 3 4 5



- 1 Molecular weight markers
- 2 PA Lysate Soluble
- 3 PA Lysate Insoluble
- 4 QAE Column Flowthrough
- 5 QAE Column Wash

Figure 14

QAE-Sephadex Purification of Propionic Acid
and Guanidine-HCl Extracted pET[(SP1)₄]/(SP2)₁₄ Protein

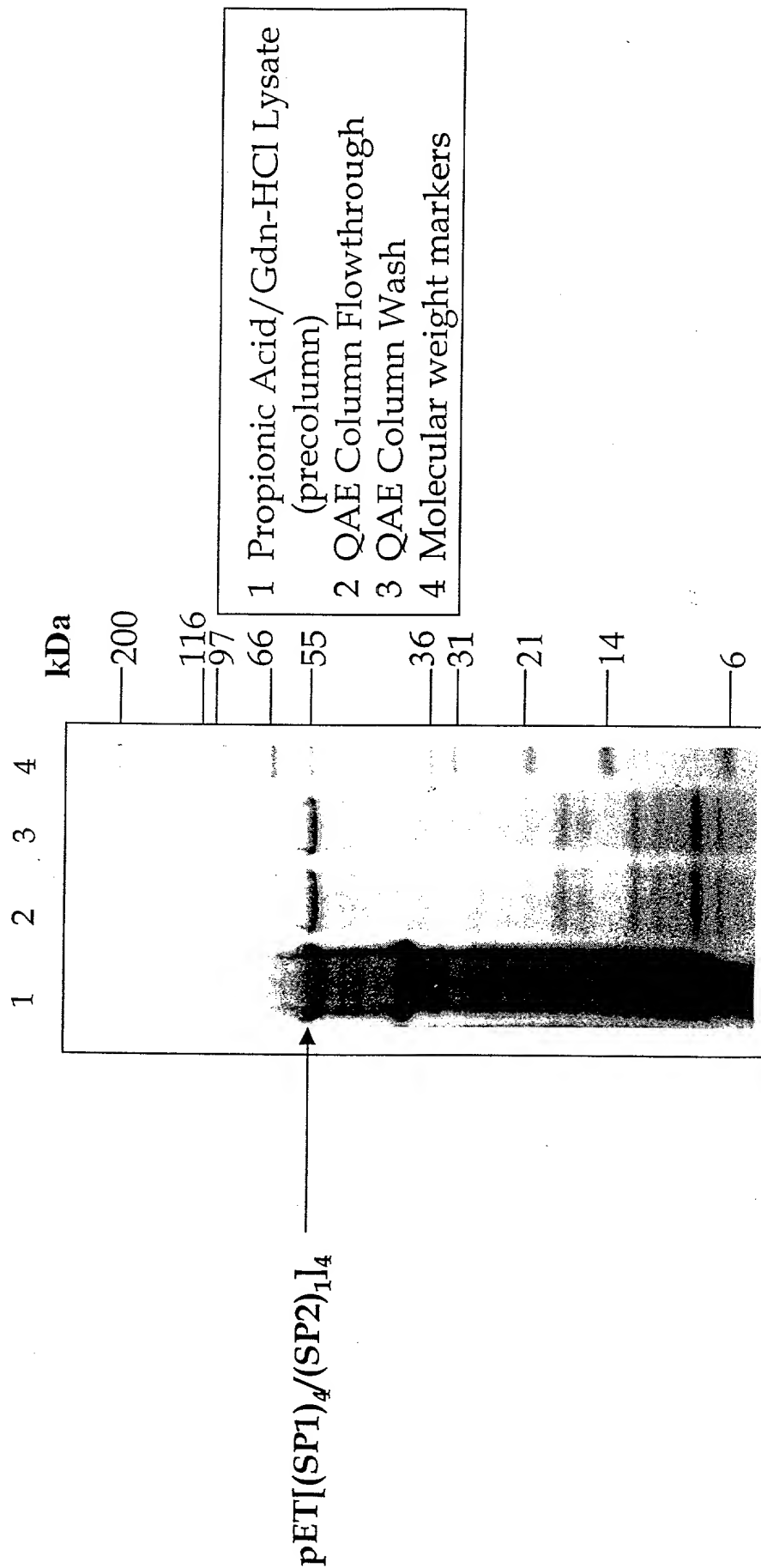


Figure 15

OmpF - Valeric Acid Lysis

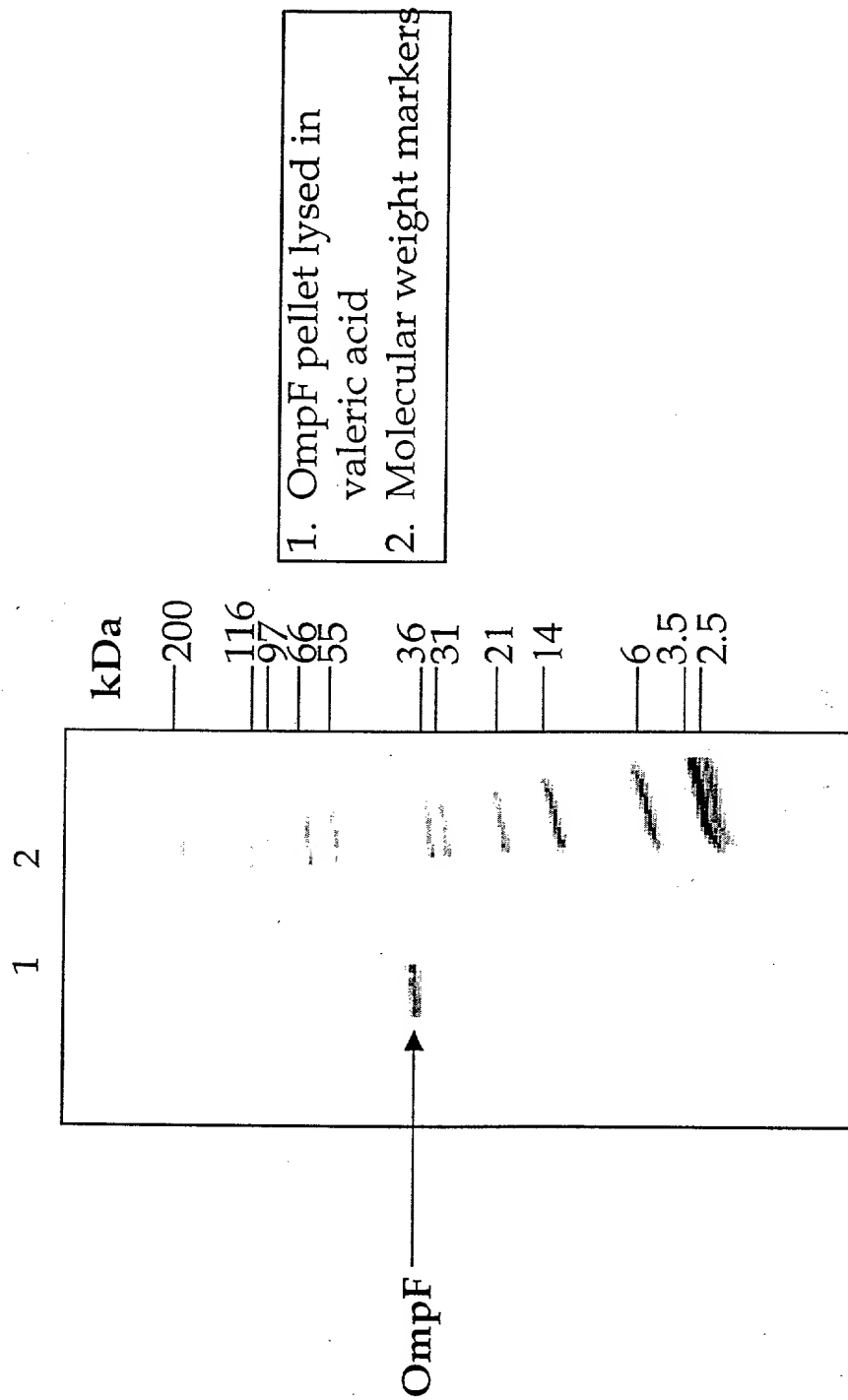


Figure 16

Comparative Gel of Recognin B1 Acid Lysis

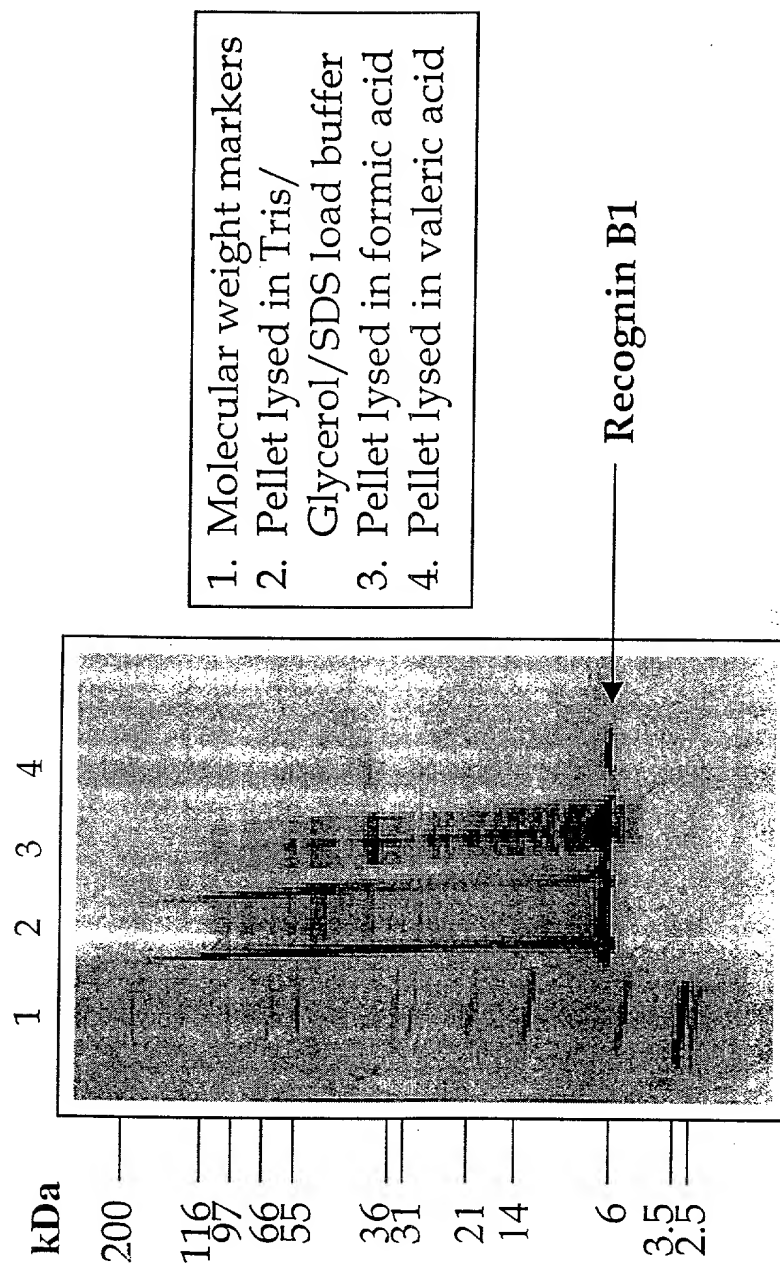
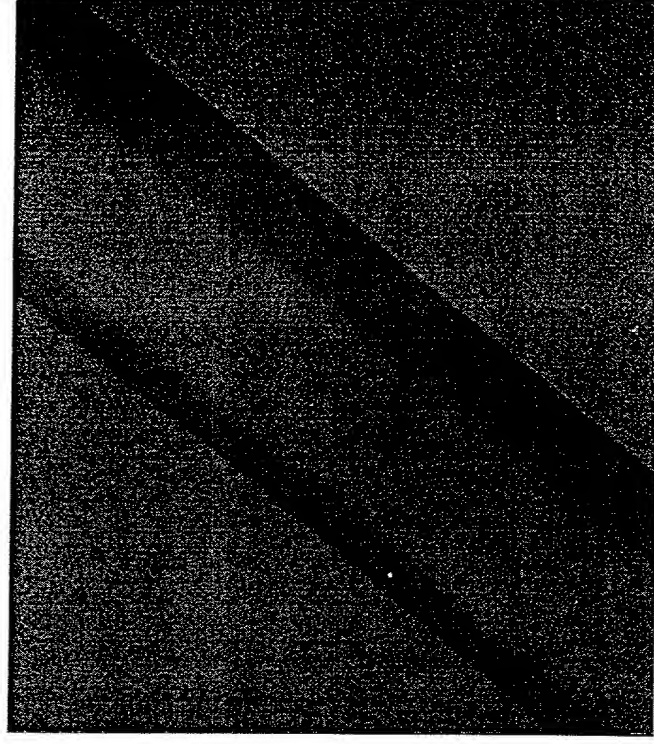
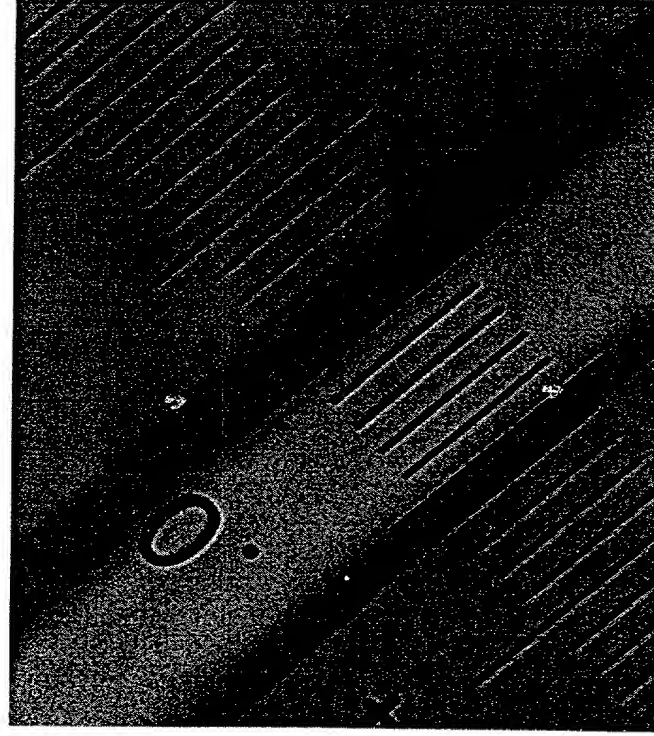


Figure 17

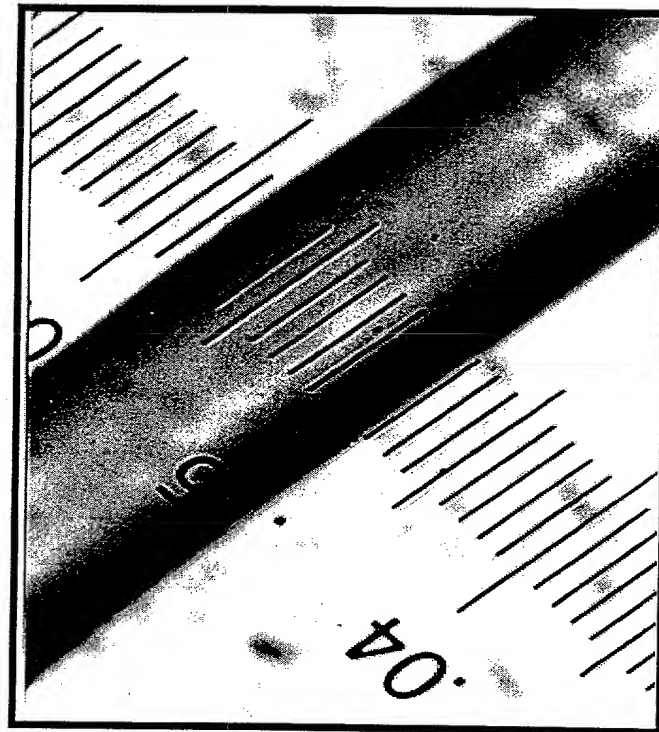
pETNcDS fiber under light microscopy. Spun from 25% protein solution into 90% methanol coagulation bath.



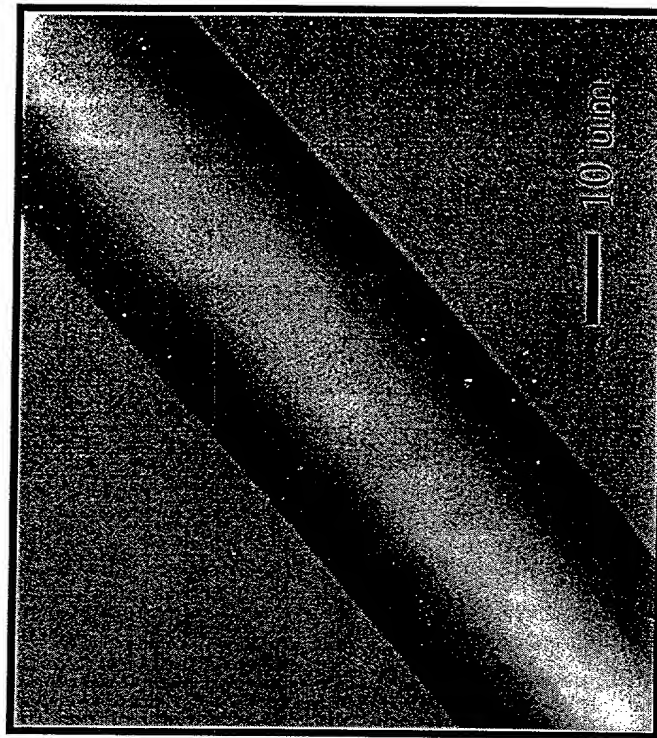
Polarized light w/tint plate

Figure 18

pQE[(SP1)₄/(SP2)₁]₄ fiber under light microscopy. Spun from a 12.5% protein solution into 90% methanol coagulation bath.



White light

Polarized light
w/ tint plate